QIFIKIT® for Quantitative Flow Cytometry

Quantification of cell surface proteins

QIFIKIT provides a flexible solution for the quantification of cell surface antigens in a variety of applications

Absolute quantification using flow cytometry can be an important tool in applications such as cellular biology, clinical research and immunotherapy research and development. QIFIKIT provides a flexible solution for quantitative flow cytometry using indirect immunofluorescence, allowing the use of any unconjugated mouse monoclonal antibody of IgG isotype.

Intended use of QIFIKIT

QIFIKIT is intended for the determination of antibody-binding and antigen density per cell by flow cytometry using indirect immunofluorescence.

The kit contains a series of beads, 10 μm in diameter and coated with different, but well-defined quantities of mouse monoclonal antibody molecules (high-affinity anti-human CD5, clone CRIS-1, isotype IgG2a). The beads mimic cells with different antigen densities which have been labeled with a primary mouse monoclonal antibody of isotype IgG. The secondary antibody binds to both the beads and the cells under investigation requiring only one calibration per experiment.

QIFIKIT contains sufficient reagents for 10 calibrations including set-up beads, calibration beads, and FITC conjugated secondary antibodies. The measurement range is approximately 2,000 - 800,000 monoclonal antibody molecules or antigenic sites per cell. For evaluation of antigen density the antibody must be used at saturation.

Principle of the assay

Sample and control labeling		Immunofluorescence staining	Flow cytometric analysis

Sample
Label cells with primary mouse mAb against antigen of interest

Isotype control
Label cells with irrelevant mouse mAb

Sample, control, set-up beads and calibration beads
Label, in parallel, with conjugated secondary anti-mouse Ab

1. Establish window of analysis (set-up beads)
2. Analyze cells and calculate ABC (sample and control)
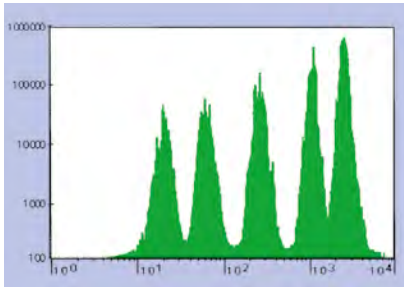3. Build calibration curve MFI against ABC (Calibration beads)

MFI: Mean Fluorescence Intensity
ABC: Antibody-Binding Capacity

Figure 1
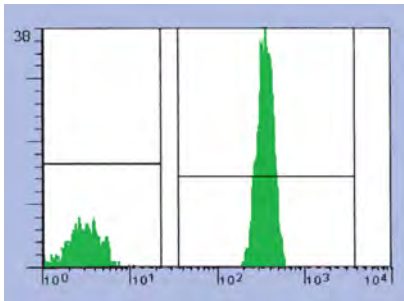Calibration Beads are used for construction of the calibration curve.

Figure 2
Quantitation of the CD4 antigen density on lymphocytes from whole blood samples using the QIFIKIT. By interpolation on the calibration curve the CD4 antigen density is found to be 57,000 sites per cell.
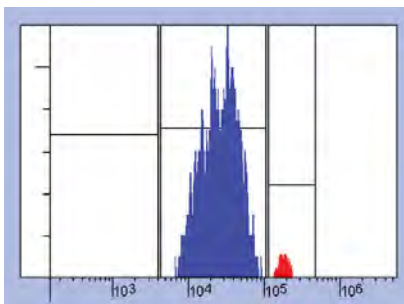

Figure 3
Determination of CD45 density on both blast cells (blue) and residual normal lymphocytes (red) from a pre-8-ALL patient using the QIFIKIT and labeling with Anti-CD45 Leucocyte Common Antigen, clone T29/33. The histogram shows a CD45 density of 200,000 sites per cell for the lymphocytes, which is in agreement with previous data (1) and 30,000 sites per cell for the leukemic population.

Advantages of using QIFIKIT

Standardization

– Gives values based on absolute quantities.

– Establish baseline antigen expression levels to serve as standard reference value.

– Enables comparisons between different laboratories and different periods of time.

– Makes quantifiable definitions of "positive" vs "negative" and "bright" vs "dim" cells possible.

Flexibility

– Allows the use of any primary unlabeled mouse monoclonal antibody of IgG isotype.

– Standards provide flexibility in instrument set-up and/or calibration.

– Applicable to cell lines, isolated cells and whole blood samples.
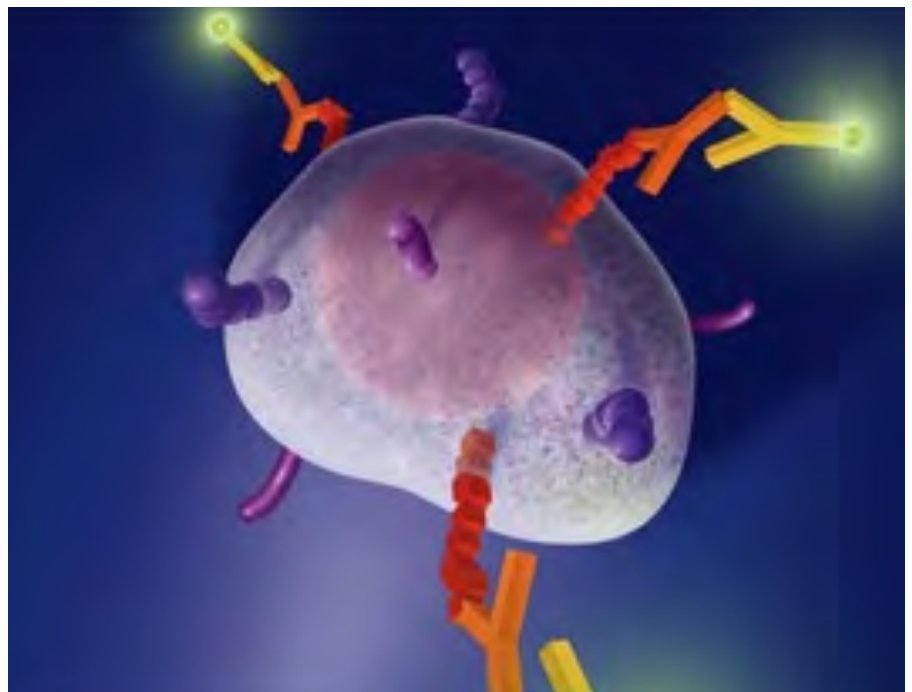
Kit Contents

Vial 1	Size
Set-Up Beads Two populations of beads. Blank beads (A) and beads with a high number of Mab molecules.	1 mL
Vial 2	
Calibration Beads Five populations of beads (B, C, D, E, and F) bearing different numbers of Mab molecules.	1 mL
Vial 3	
FITC Conjugate F(ab')$_2$ Fragment of FITC-Conjugated Goat Anti-Mouse Immunoglobulins (affinity-isolated). FITC conjugate for staining of set-up beads, calibration beads and up to 80 samples.	0.2 mL

Ordering Information

Product	Size	Code
QIFIKIT	10 calibrations	K007811-8

For research use only. Not for diagnostic procedures.

References

1. Lavabre-Bertrand T, et al. Quantification of CD24 and CD45 antigens in parallel allows a precise determination of B-cell maturation stages: relevance for the study of B-cell neoplasias. Leukemia 1994;8:402-8.
2. Olejniczak, Scott H., et al. A Quantitative Exploration of Surface Antigen Expression in Common BCell Malignancies Using Flow Cytometry, Immunological Investigations, 2006;35:1, 93-114.
3. Wasiluk, A. Platelet expression of CD62P in hypotrophic newborns, Platelets, 2012;23:2, 161-165.
4. Battle, R., Quantitative analysis of human leucocyte antigen expression during culture of Epstein-Barr virustransformed cell lines using the Dako QIFIKIT, British Journal of Biomedical Science, 2007;64:1, 32-34

Learn more:
www.agilent.com

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This information is subject to change without notice.

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